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L3: Entry 1 of 1

File: USPT

Jun 19, 2001

DOCUMENT-IDENTIFIER: US 6248874 B1

TITLE: DNA molecules encoding bacterial lysine 2,3-aminomutase

Brief Summary Text (45):

4. Isolation of Cloned Lysine 2,3-Aminomutase and Production of Anti-Lysine 2,3-Aminomutase Antibodies

Brief Summary Text (58):

Additional variations in purification are described by Petrovich et al., J. Biol. Chem. 226:7656 (1991), and can be devised by those of skill in the art. For example, anti-lysine 2,3-aminomutase antibodies, obtained as described below, can be used to isolate large quantities of lysine 2,3-aminomutase by immunoaffinity purification.

Brief Summary Text (64):

(b) Preparation of Anti-Lysine 2,3-Aminomutase Antibodies and Fragments Thereof

Brief Summary Text (66):

Alternatively, an anti-lysine 2,3-aminomutase antibody can be derived from a rodent monoclonal antibody (MAb). Rodent monoclonal antibodies to specific antigens may be obtained by methods known to those skilled in the art. See, for example, Kohler et al., Nature 256:495 (1975), and Coligan et al. (eds.), CURRENT PROTOCOLS IN IMMUNOLOGY, VOL. 1, pages 2.5.1-2.6.7 (John Wiley & Sons 1991) ["Coligan"]. Also see, Picksley et al., "Production of monoclonal antibodies against proteins expressed in E. coli," in DNA CLONING 2: EXPRESSION SYSTEMS, 2nd Edition, Glover et al. (eds.), pages 93-122 (Oxford University Press 1995).

Brief Summary Text (69):

For particular uses, it may be desirable to prepare fragments of anti-lysine 2,3-aminomutase antibodies. Such antibody fragments can be obtained, for example, by proteolytic hydrolysis of the antibody. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. As an illustration, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')₂. This fragment can be further cleaved using a thiol reducing agent to produce 3.5S Fab' monovalent fragments. Optionally, the cleavage reaction can be performed using a blocking group for the sulfhydryl groups that result from cleavage of disulfide linkages. As an alternative, an enzymatic cleavage using pepsin produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. Pat. Nos. 4,036,945 and 4,331,647 and references contained therein. Also, see Nisonoff et al., Arch Biochem. Biophys. 89:230 (1960); Porter, Biochem. J. 73:119 (1959), Edelman et al., in METHODS IN ENZYMOLOGY VOL. 1, page 422 (Academic Press 1967), and Coligan at pages 2.8.1-2.8.10 and 2.10.-2.10.4.

Brief Summary Text (87):

Anti-lysine 2,3-aminomutase antibodies can also be used to isolate DNA sequences that encode enzymes from cDNA libraries. For example, the antibodies can be used to screen λ gt11 expression libraries, or the antibodies can be used for

immunoscreening following hybrid selection and translation. See, for example, Ausubel et al. (eds.), SHORT PROTOCOLS IN MOLECULAR BIOLOGY, 3rd Edition, pages 6-12 to 6-16 (John Wiley & Sons, Inc. 1995); and Margolis et al., "Screening .lambda. expression libraries with antibody and protein probes," in DNA CLONING 2: EXPRESSION EXPRESSION SYSTEMS, 2nd Edition, Glover et al. (eds.), pages 1-14 (Oxford University University Press 1995).

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☐ 2. 20030202989. 08 Apr 99. 30 Oct 03. USE OF TOXIN PEPTIDES AND/OR AFFINITY HANDLES FOR DELIVERING COMPOUNDS INTO CELLS. COLLIER, R. JOHN, et al. 424/236.1; 435/252.3 435/320.1 435/69.7 514/12 530/350 536/23.7 A61K039/02 C12P021/04 C12N001/21 C07K014/195 C07H021/04 C12N015/74.

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☐ 32. US20030108556A. Treatment of mammal suffering from or susceptible to infectious agent involves administering polymer having polymerized dextran units or polymerized ethylene glycol units linked to several therapeutic agents. COLLIER, R J, et al. A61K039/00 A61K039/38.

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☐ 39. WO 9723236A. Introducing therapeutic proteins, especially antigens, into cells - using toxin molecules and/or polycationic handles for delivery. BALLARD, J D, et al. A61K038/00 A61K038/07 A61K039/00 A61K039/02 A61K039/04 A61K039/112 A61K039/12 A61K039/13 A61K039/165 A61K039/20 A61K039/205 A61K039/21 A61K039/245 A61K039/25 A61K039/285 A61K039/29

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☐ 43. EP 643559B. Polypeptide(s) corresp. to diphtheria toxin receptor binding region - used for treating diphtheria or immunising against diphtheria toxin. CHOE, S, et al. A01N037/18 A01N063/00 A61K037/00 A61K038/00 A61K039/05 A61K039/40 A61K049/00 A61P031/04 C07H017/00 C07H019/00 C07H021/00 C07K001/02 C07K001/12 C07K003/00 C07K013/00 C07K014/34 C07K015/00 C07K017/00 C07K019/00 C12N001/20 C12N001/21 C12N005/00 C12N015/00 C12N015/09 C12P021/02 C12P021/06 C12N001/21 C12R001:19 C12P021/02 C12R001:19.

☐ 44. US 4709017A. Modified diphtheria toxin fragment-A and vaccine - has no ADP-ribose transfer activity and is immunologically cross-reactive with natural fragment-A. CARROLL, S F, et al. C07K013/00.

☐ 45. EP 44167A. Target-specific cytotoxic agents - comprising antibody linked to enzymatically active toxin fragment. COLLIER, R J, et al. A61K035/74 A61K039/39.

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TITLE: Analyte sensing mediated by adapter/carrier molecules

Detailed Description Text (127):

The following proteins were used in Examples 9-11. The mutant .alpha.HL genes M113N, M113N/L135N and E111N/K147N were prepared by cassette mutagenesis in the plasmid .alpha.HL-RL2 (Cheley, S., et al., Protein Sci., 8:1257-1267, 1999). These constructs contain the following additional changes over WT-.alpha.HL: Lys-8->Ala, Val-124->Leu, Gly-130->Ser, Asn-139->Gln, Ile-142->Leu. .alpha.HL polypeptides with these mutations behave similarly to WT-.alpha.HL in hemolysis assays and in planar bilayer recordings, at the salt concentrations used herein (Cheley, S., et al., Protein Sci., 8:1257-1267, 1999). .alpha.HL-CH1 is one of several chimeric proteins that feature a transmembrane domain derived from the protective antigen of anthrax toxin fused to the cap domain of .alpha.HL (laboratories of R. J. Collier and H. B., in preparation). Residues 119-140 inclusive of .alpha.HL (21 residues) were replaced with 22 residues 302-323 from protective antigen. The register of the .beta. strands in the transmembrane domain is that given by Petosa and colleagues (Petosa, C., et al., Nature, 385:833-838, 1997).

Detailed Description Text (153):

Because the increase in anion selectivity observed when .beta.CD was used as an adapter for the WT-.alpha.HL pore was modest, in this example it is determined whether .beta.CD would produce anion selectivity in a cation-selective pore. To this end, .alpha.HL-CH1, a chimeric protein that features a transmembrane domain derived from the protective antigen of anthrax toxin fused to the cap domain of .alpha.HL, was examined. The net charge per subunit in the transmembrane barrel of homoheptameric .alpha.HL-CH1 is -21, compared with -7 in the WT-.alpha.HL barrel, and it is cation selective. The altered barrel in .alpha.HL-CH1 retains the site near Met-113, where cyclodextrins are believed to bind (Gu, L.-Q., et al., Nature, 398:686-690, 1999). Once again, permeability ratios were determined from V.sub.r values (FIGS. 9a, b).

Other Reference Publication (30):

Petosa, et al., "Crystal structure of the anthrax toxin protective antigen," Nature, vol. 385, pp. 833-838 (Feb. 27, 1997).

CLAIMS:

2. A system for sensing a plurality of different analytes comprising: at least one sensor element, each sensor element comprising a pore and having a receptor site; and a plurality of different host molecules, wherein the host molecules each interact with a receptor site of a sensor element and at least one of the different analytes as an adapter between the analyte and the receptor site so that the sensor element directly produces a detectable signal.

5. A system for sensing a plurality of different analytes comprising: a plurality of different sensor elements, each sensor element comprising a pore and having a

receptor site; and a plurality of different host molecules, wherein the host molecules each interact with a receptor site of one of the plurality of different sensor elements and one of the different analytes as a carrier to deliver the analyte to the receptor site so that the sensor element directly produces a detectable signal.

21. The system of claim 20 wherein the protein is selected from the group consisting consisting of a transmembrane pore, an enzyme, an antibody and a receptor.

22. The system of any one of claim 1 or 4 wherein the sensor element comprises a pore.

23. The system of claim 22 wherein the sensor element comprises a genetically engineered transmembrane protein pore.

24. The system of claim 22 wherein the sensor element is an .alpha.-Hemolysin (.alpha.HL) pore.

25. The system of claim 24 wherein the sensor element is a wild-type .alpha.-Hemolysin (.alpha.HL) pore.

26. The system of claim 24 wherein the sensor element is a genetically engineered or mutant .alpha.-Hemolysin (.alpha.HL) pore.

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